# *Molecular distribution of wastewater from a meat by-products processing company, treated using a batch system (SBR)*

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*Distribución molecular del agua residual de una empresa de procesamiento de subproductos cárnicos tratada mediante un sistema discontinuo (SBR)*

*Distribució molecular de l'aigua residual d'una empresa de processament de subproductes carnis tractada mitjançant un sistema discontinu (SBR)*

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## RESUMEN

Los tratamientos biológicos de aguas residuales, dependen de las características de la materia orgánica. El fraccionamiento en pesos moleculares, de los diferentes componentes de las aguas residuales, es de gran importancia ya que ayuda al diseño de tecnologías de tratamiento apropiadas. El propósito de este estudio fue evaluar la distribución de los tamaños de las partículas presentes en dos tipos de aguas residuales generadas en una empresa de procesamiento de subproductos cárnicos y sus respectivas mezclas, previo y posterior al tratamiento mediante un sistema en discontinuo Sequencing Batch Reactor (SBR). Los análisis en los afluentes del sistema mostraron que la mayor parte de los parámetros analizados tenían un peso molecular mayor a 10,000 Da. Los resultados de los efluentes demostraron que el sistema SBR es adecuado para remover una alta fracción de materia orgánica fácilmente biodegradable, alcanzándose remociones del orden de 98.68%, 98.14% y 70.51% para la DQO, $DBO_5$ y $N\text{-}NH_4^+$ respectivamente.

***Palabras clave:*** Sistema de lotes, aguas residuales industriales, los productos cárnicos, la distribución del peso molecular.

## SUMMARY

Biological treatments of wastewater depend on the characteristics of organic matter. The molecular weight fractionation of the various components of wastewater is of great importance as it helps to design appropriate treatment technologies. The purpose of this study was to evaluate the distribution of particle sizes present in two types of wastewater generated in a company that processes meat by-products and their respective mixtures. Evaluation was carried out before and after treatment using a discontinuous system in a Sequencing Batch Reactor (SBR). The analysis in the tributaries of the system showed that most of the parameters analyzed had a molecular weight higher than 10,000 Da. The results of the effluents showed that the SBR system is adequate to remove a high fraction of readily biodegradable organic matter, achieving a removal order of 98.68%, 98.14% and 70.51% for COD, $BOD_5$ and $NH_4^+\text{-}N$ respectively.

***Keywords:*** Batch system; Industrial wastewater; meat products; molecular weight distribution.

## RESUM

Els tractaments biològics d'aigües residuals, depenen de les característiques de la matèria orgànica. El fraccionament en pesos moleculars, dels diferents components de les aigües residuals, és de gran importància ja que ajuda al disseny de tecnologies de tractament apropiades. El propòsit d'aquest estudi va ser avaluar la distribució de les mides de les partícules presents en dos tipus d'aigües residuals generades en una empresa de processament de subproductes carnis i les seves respectives mescles, prèvia i posterior al tractament mitjançant un sistema en discontinu Sequencing Batch Reactor (SBR). Les anàlisis dels afluents del sistema van mostrar que la més gran que part dels paràmetres analitzats tenien un pes molecular més gran que 10,000 Da. Els resultats dels efluents van demostrar que el sistema SBR és adequat per eliminar una fracció elevada de matèria orgànica fàcilment biodegradable, aconseguint-eliminacions de l'ordre del 98.68%, 98.14% i 70.51% per la DQO, $DBO_5$ i $N\text{-}NH_4^+$ respectivament.

***Mots clau:*** Sistema de lots, aigües residuals industrials, els productes carnis, la distribució del pes molecular

# 1.INTRODUCTION

The size distribution of pollutants has been widely used for the interpretation of residual water characteristics, technology assessment, appropriate treatment and estimation of removals. The particles in wastewater have been grouped into categories by size, known as dissolved components (<1 nm), colloidal components (1-$10^3$ nm), supracoloidales components ($10^3$-$10^5$ nm) and settleable components (>$10^5$ nm). Recently, ultrafiltration, among other methods, has been successfully used to identify and differentiate pollutants in wastewater within narrower ranges (Dogruel et al., 2006, Engstrom and Gytel, 2000; Sophonsiri and Morgenroth, 2004). Therefore, research efforts have been directed towards the use of information regarding the size of the particles in order to understand and thereby improve biological processes (Sophonsiri and Morgenroth, 2004).

Different organic matter fractions are distinguished in the rates of biodegradation. Chemical oxygen demand (COD) as a way to classify organic matter was used for the first time in the bio substrate model for activated sludge processes introduced by Dold et al., 1980. According to this model, the total COD of the effluent has three main components: the total biodegradable COD ($COD_{TB}$), the total non-biodegradable COD ($COD_{NTB}$) and the active biomass COD (Orhon and Okgor, 1997; Wentzel et al., 1999). The most commonly used fraction in the design, control and modeling of activated sludge processes for nutrient removal is the $COD_{TB}$. In the model presented by Dold et al., 1980, this fraction is subdivided into readily biodegradable ($COD_{EB}$) and slowly biodegradable ($COD_{SB}$) components.

The $COD_{EB}$ is composed of relatively small biodegradable particles, which are easily transported across the cell membrane and are metabolized within minutes. (Dulekgurgen et al., 2006) These particles include volatile fatty acids (VFA), carbohydrates simple alcohols and amino acids, which can be directly absorbed into the cell. Acetic acid is the main component of the VFA. $COD_{EB}$ consists of 8 to 25% of the total COD in wastewater and 10 to 35% of that in sedimented residual water (Wentzel et al., 1999).

The $COD_{SB}$ fraction takes longer to degrade, as the particles are larger and require extracellular hydrolysis before being transported into the cells (Hu et al., 2002; Wentzel et al., 1999). This fraction has been considered fundamental in the design and operation of nitrogen and phosphorus removal systems (Tasli et al., 1999). The elimination rate of these two nutrients is highly dependent on the concentration of the readily biodegradable fraction.

In accordance with the classification of the two $COD_{TB}$ fractions, filtration methods using filters with a variety of pore sizes have been proposed and applied (Bortone et al., 1993; Mamais et al., 1993).

The Sequencing Batch Reactor (SBR) is a biological treatment system characterized by using a series of consecutive steps in time, for example: filling, reaction, sedimentation, discharge, bleeding and inactivity, all carried out within the same tank (Gali et al., 2008). Nitrogen removal in SBR systems can be achieved by alternating aerobic and anoxic periods during the reaction, allowing the nitrogen cycle to be completed (Mahvi, 2008).

Several authors have studied different operational strategies to remove organic matter, nitrogen and phosphorus in SBR reactors (Table 1). The SBR system performance is generally comparable to a conventional activated sludge system and depends on the system design criteria. Based on various studies, average efficiencies of between 89-98% for $BOD_5$, 91-95% for COD, and > 75% for total ni-

***Table 1.*** *Comparison of the different types of effluent treated with the SBR system*

| Type of effluent | HRT (d) | BOD5 (%) | COD (%) | TN (%) | TNK (%) | Reference |
|---|---|---|---|---|---|---|
| Wastewater from poultry slaughterhouse | - | - | 93 | - | - | Yu et al., 1997 |
| Wastewater from pig slaughterhouse | - | - | 93 | 90 | - | Bortone et al., 1993 |
| Industrial wastewater | - | 85 | - | - | - | Lim et al., 2002 |
| | - | 99 | - | - | - | Lin and Jiang, 2003 |
| | - | 82 | 91 | - | 63 | Sirianuntapiboon and Ungkaprasatcha, 2007 |
| | - | 80 | - | - | - | Ammary, 2005 |
| | - | 80 | - | 38 | 75 | Li and Zhand, 2002 |
| | - | 80 | - | - | - | Ammary, 2005 |
| | - | - | - | - | - | Schwarzenbeck et al., 2004 |
| | - | 85 | 95 | - | - | Goncalves et al., 2005 |
| | - | - | - | - | - | Keller et al., 1997 |
| | - | 80 | - | - | 80 | Li and Zhand, 2002 |
| | 1.1 | - | - | - | 95 | Andreottola et al., 2001 |
| | 4.4 | - | - | - | 60 | Garrido et al., 2001 |
| | 1.7 | - | - | - | 23 | Villaverde et al., 2000 |
| | 44.0 | - | - | - | 93 | Choi et al., 1997 |
| | - | - | - | - | 74 | Kabacinski et al., 1998 |
| | 0.8 | - | - | - | 97 | Keller et al., 1997 |
| | 1.0 | - | - | - | 99 | Obaja et al., 2003 |
| | 10.0 | - | - | - | 98 | Tilche et al., 1999 |

trogen have been found in SBR systems, depending on the biodegradability of the compounds present in wastewater. This study used a SBR pilot system for the removal of organic matter and nitrogen from wastewater from a meat by-products processing company. The system was monitored in each of its eight stages by analyzing the molecular weight distribution of both the influent and the treated effluent.

## 2. MATERIALS AND METHODS

### 2.1Pilot-scale SBR reactor

The reactor have a working volume of 2.96 $m^3$was equipped with a submersible aerator (AQUA 200) generating an intermittent aeration, a submersible pump (NOVA 180) operating as a clarified water pump of excess sludge and of the reactor supply and an agitation system that was connected to the aeration pump. The SBR operation cycles were controlled by a programmable PLC (ATB to 230V). The phases carried out by the reactor in each cycle were: filling, reaction, settle and draw, with duration of 480 min in a complete cycle.
Two types of wastewater were studied: water from the washing of vehicles, equipment, barrels and packages containing meat products, and condensed water generated after transforming the raw material into flour and fat for animal feed using the cooker process. The characterization of both types of wastewater is presented in Table 2.

***Table 2.*** *Characterisation of the washing water and condensed water used for the study; the standard deviation was calculated with n =10*

| PARAMETER | UNITS | WASHING WATER | CONDENSATE WATER |
|---|---|---|---|
| | | Average ± Std. Dev. | Average ± Std. Dev. |
| $COD_T$ | mg/L | 8308.33 ± 1823.38 | 1381.14 ± 483.64 |
| $COD_F$ | mg/L | 3922.44 ± 1539.83 | 822.47 ± 215.33 |
| $BOD_5$ | mg/L | 2684.54 ± 1686.49 | 563.42 ± 219.39 |
| TSS | mg/L | 1710.70 ± 973.84 | 7.19 ± 3.12 |
| VSS | mg/L | 1242.15 ± 817.43 | 6.13 ± 2.91 |
| $NH_4^+$-N | mg/L | 365.14 ± 85.66 | 615.54 ± 129.39 |
| pH | - | 6.11 ± 0.40 | 9.64 ± 0.47 |
| $BOD_5/NH_4^+$-N | - | 7.45 ± 4.59 | 0.94 ± 0.36 |

The experiments were divided into eight stages in which the mixing proportions of both the washing water and the condensate were varied (Table 3). Samples were taken during the 252 days that the reactor was operated.

***Table 3.*** *Operational steps of the SBR system*

| Stages | Time of Operation (d) | Mix ratio | |
|---|---|---|---|
| | | Washing water (%) | Condensate water (%) |
| I | 0 - 21 | 100 | 0 |
| II | 22 - 43 | 100 | 0 |
| III | 44 - 77 | 100 | 0 |
| IV | 78 - 98 | 90 | 10 |
| V | 99 - 154 | 80 | 20 |
| VI | 155 - 189 | 50 | 50 |
| VII | 190 - 231 | 30 | 70 |
| VIII | 232 - 252 | 0 | 100 |

### 2.2 Fracionation

Ultrafiltration tests were conducted according to the protocols established by Dulekgurgen et al., 2006 and Rodriguez et al., 2011, using a stirring cell (Amicon, model 8400) with a working volume of 400 ml, a filter diameter of 76 mm and a filtration area of 41.8 $cm^2$ (Figure 1). The samples were taken both in the influent and in the effluente of the SBR reactor a different times in each of the eight phases of operation of the system and sequentially filtered through cellulose membranes (PL and YM Ultracel series, Millipore) with nominal molecular weights (MWCO) of 10,000, 5,000 and 1,000 Da. Once the sample was inside the system, throughout the experiment nitrogen was passed to the stirring cell with a pressure of 15 psi. Prior to ultrafiltration, the pH of the sample was adjusted to 8.0 and the sample was filtered using membranes of 0.45 mm to remove as much of the suspended solids as possible to prevent it from interfering with the ultrafiltration.
After ultrafiltration, four samples were obtained: the first was retained in the 10,000 Da membrane and was designated "MW >10,000". The second passed through the 10,000 Da membrane but was retained in that of 5,000 Da and was designated "10,000< MW <5,000". The third passed through the 5,000 Da membrane, but was retained in that of 1,000 Da and was designated "5,000< MW <1,000" Finally, the fourth sample passed through the 1,000 Da membrane and was designated "MW <1,000" (Rodriguez et al., 2011). Ultrafiltration tests were conducted on samples of influent and effluent at each stage of operation of the SBR, in order to determine the distribution of particles in terms of COD, $BOD_5$, $NH_4^+$-N and proteins.

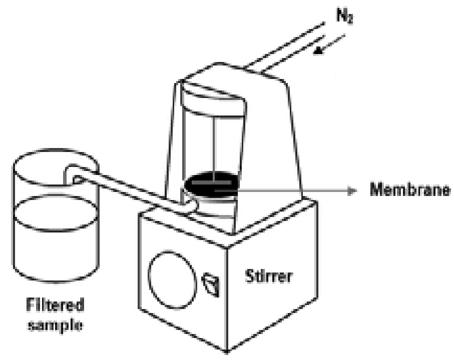



***Figure 1.*** Stirrer cell used for the ultrafiltration test

### 2.3 Chemical Analysis

Various parameters such as Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand ($BOD_5$) were evaluated in accordance with protocols established in Standard Methods (APHA, 2005). Ammoniacal nitrogen ($NH_4^+$-N) was evaluated using Kjeldahl nitrogen equipment (Bυchi, 100% steam, distillation time 5 min). The proteins were determined colorimetrically based on the Lowry method (Lowry et al., 1951), as modified by Peterson (Peterson, 1977).

## 3. RESULTS

### 3.1 Removals

During phase IV, removals reached 98.68, 98.14 and 70.51% for COD, $BOD_5$ and $NH_4^+$-N, respectively, corresponding to a ratio of washing water: condensate water of 90%: 10%. The lowest removals for COD, $BOD_5$ and $NH_4^+$-

N were obtained during phase I, during which the SBR reactor operated only with washing water.

***Table 4.*** *Removal percentages in each of the stages*

| Stages | Removal (%) | | |
|---|---|---|---|
| | COD | $BOD_5$ | $NH_4^+$-N |
| I | 64.35 ± 3.69 | 66.47 ± 5.78 | 29.39 ± 5.14 |
| II | 74.69 ± 5.43 | 79.26 ± 4.12 | 49.69 ± 6.83 |
| III | 89.85 ± 7.25 | 91.33 ± 6.95 | 56.25 ± 8.09 |
| IV | 98.68 ± 0.45 | 98.14 ± 0.47 | 70.51 ± 7.56 |
| V | 88.76 ± 0.61 | 90.91 ± 2.70 | 19.18 ± 2.64 |
| VI | 97.57 ± 1.33 | 94.54 ± 0.99 | 60.89 ± 7.80 |
| VII | 82.94 ± 4.20 | 80.50 ± 2.32 | 40.98 ± 2.08 |
| VIII | 74.72 ± 8.28 | 76.78 ± 5.26 | 58.52 ± 1.89 |

### 3.2 Stage I

In the influent of phase I (Figure 2), the highest fractions of the different compounds measured were greater than 10,000 Da in all cases, with retention rates of 64%, 65 %, 39% and 95% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively. In the effluent fractions of all the compounds were very evenly distributed throughout the four sizes of membrane. However, the highest retention rates were 50% for COD with a membrane size larger than 10,000 Da, 32% for $BOD_5$ (5,000-1,000 Da), 33% for $NH_4^+$-N (5,000-1,000 Da) and 50% for proteins (<1,000 Da).

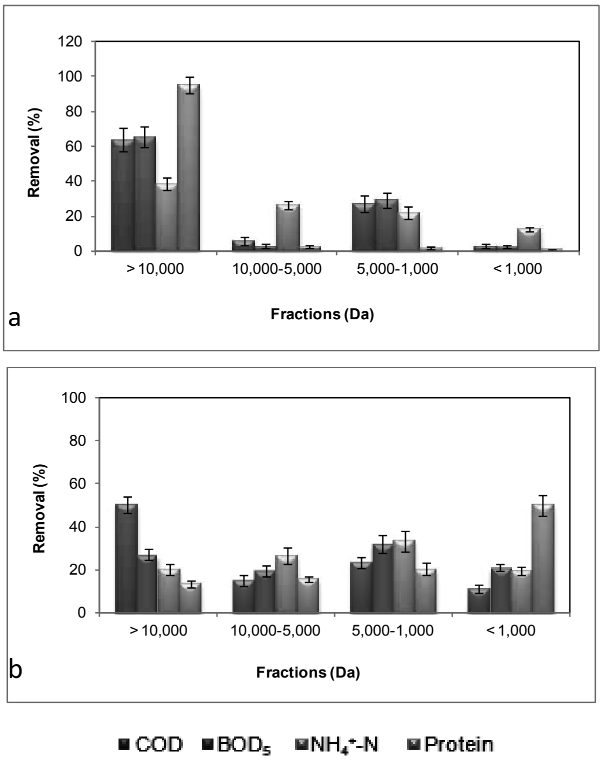



***Figure 2.*** *Ultrafiltration results of wastewater for stage I (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.3 Stage II

In the influent of phase II (Figure 3), as in stage I, the highest fractional parts of the different compounds measured were in the membrane size greater than 10,000 Da in all cases (COD, $BOD_5$, $NH_4^+$-N and proteins), with retention rates of 90%, 88%, 47% and 97% respectively. In the effluent, fractions of all compounds were also distributed throughout the four sizes of membrane. However, the highest retention rates were 37% for COD, 39% for $BOD_5$ and 33% for $NH_4^+$-N, all with a membrane size between 5,000-1,000 Da. In the case of proteins, greater retention occurred in the >10,000 Da fraction, with a rate of 79%.

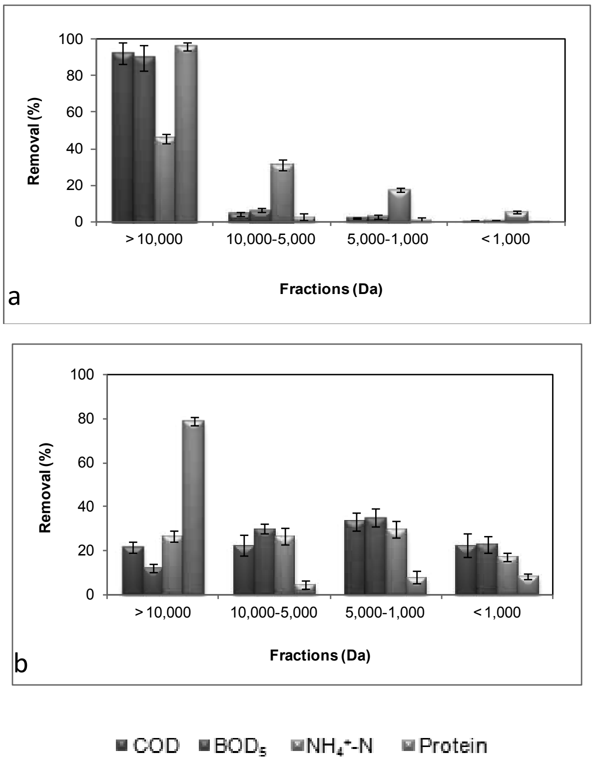
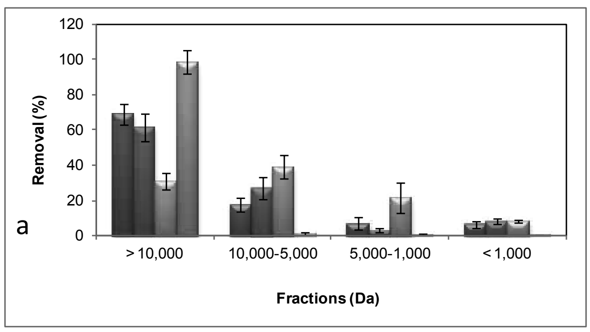



***Figure 3.*** *Ultrafiltration results of wastewater for stage II (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.4 Stage III

In Stage III, the highest retention rates for the influent were 69%, 61% and 99% for COD, $BOD_5$ and proteins respectively, all in the >10,000 Da fraction. In the case of $NH_4^+$-N, the greatest retention was in the 10,000 to 5,000 Da fraction with a rate of 40% (Figure 4). In contrast, in the effluent the highest retentions of all compounds were present in the <1,000 Da fraction, with percentages of 78%, 81%, 77 % and 55% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively.



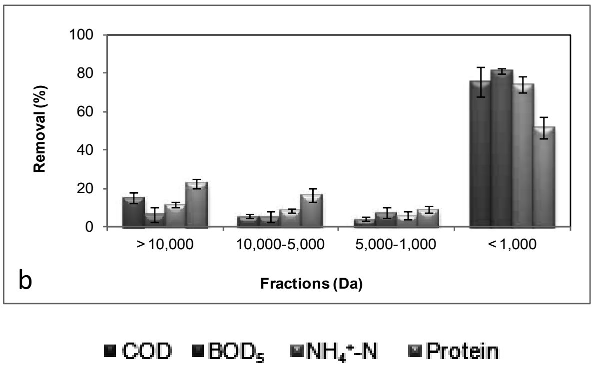



***Figure 4.*** *Ultrafiltration results of wastewater for stage III (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.5 Stage IV

In the influent of stage IV, the highest retention rates were in the >10,000 Da fraction with 68%, 60% and 43% and 97% for COD, $BOD_5$, $NH_4^+$-N and proteins, respectively (Figure 5) . In the effluent, as in stage III, the largest fraction of all the compounds analyzed was <1,000 Da with percentages of 81%, 80%, 69% and 42% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively.

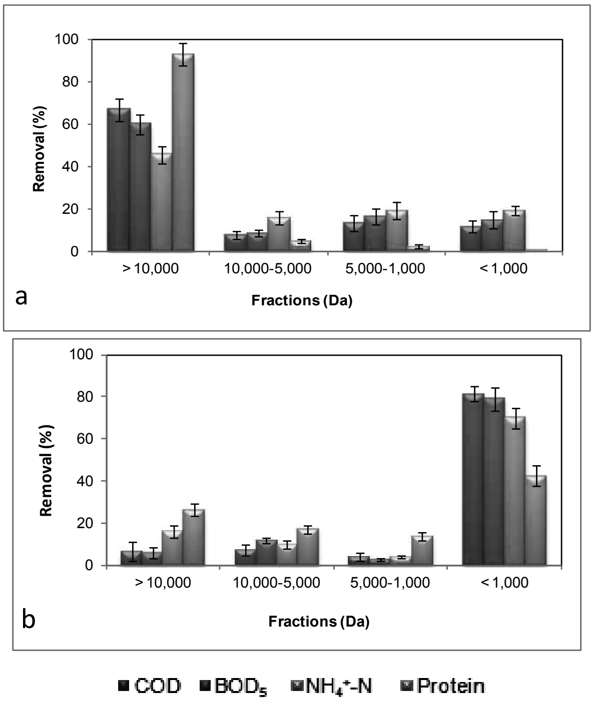



***Figure 5.*** *Ultrafiltration results of wastewater for stage IV (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.6 Stage V

In the influent of stage V, as in previous stages, the highest retention rates were in the >10,000 Da fraction with values of 84%, 66%, 61% and 92% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively (Figure 6). In the effluent, as in stages I and II, the compounds were distributed in all fractions. However, the highest retentions were 75% for COD in the >10,000 Da fraction and for the $BOD_5$, $NH_4^+$-N and proteins, percentages of 35%, 34% and 34% respectively, in the 10,000-5,000 Da fraction.

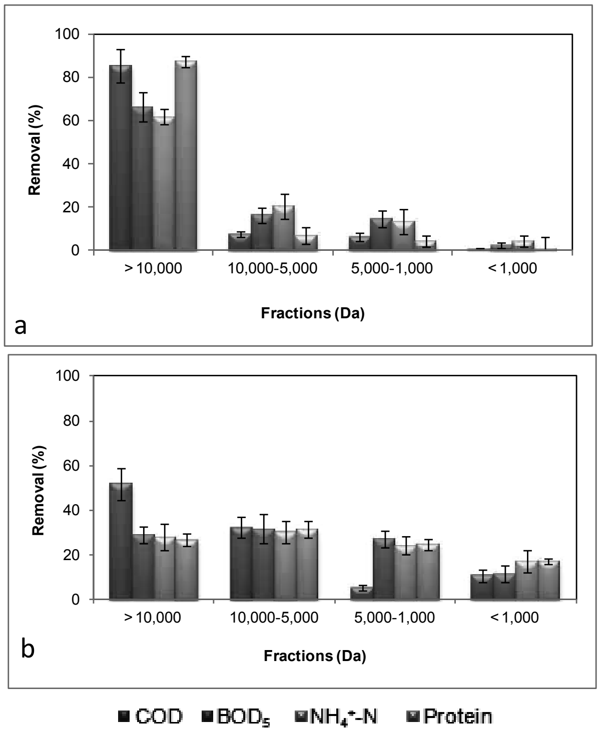



***Figure 6.*** *Ultrafiltration results of wastewater for stage V (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.7 Stage VI

In the influent in phase VI, the highest retention rates were in the >10,000 Da fraction with values of 77%, 78%, 43% and 98% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively (Figure 7). In the effluent, as in stages III and IV, the largest fraction of all the compounds analyzed was <1,000 Da with percentages of 59%, 63%, 80% and 39% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively.

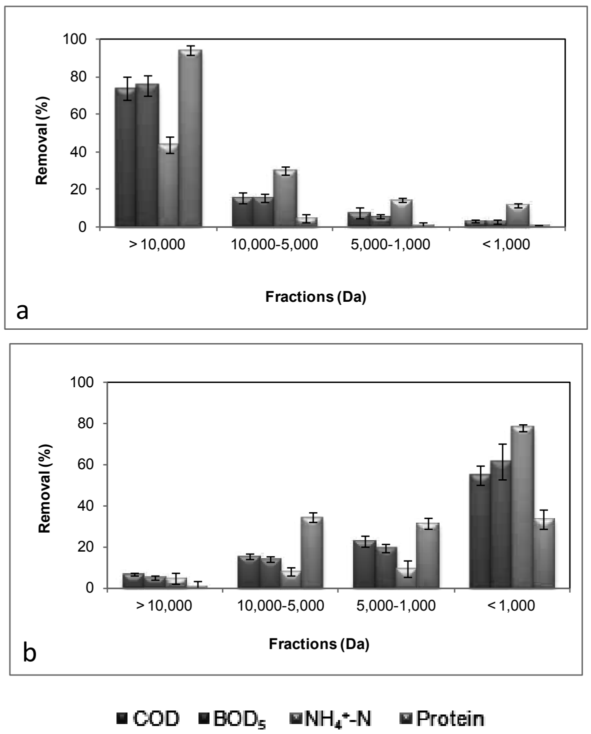



***Figure 7***. *Ultrafiltration results of wastewater for stage VI (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.8 Stage VII

In the influent in phase VII, the highest retention rates were in the >10,000 Da fraction for COD, $BOD_5$ and proteins, with values of 93%, 57% and 95%, respectively. In the case of $NH_4^+$-N, the greatest retention occurred in the 10,000-5,000 Da fraction with a value of 47% (Figure 8). In the effluent the same pattern occurred as in the influent for COD, $BOD_5$ and proteins, with percentages of 53%, 46% and 73% respectively, in the >10,000 Da fraction. The greatest retention for $NH_4^+$-N occurred in the 5,000-1,000 Da fraction, at a rate of 37%.

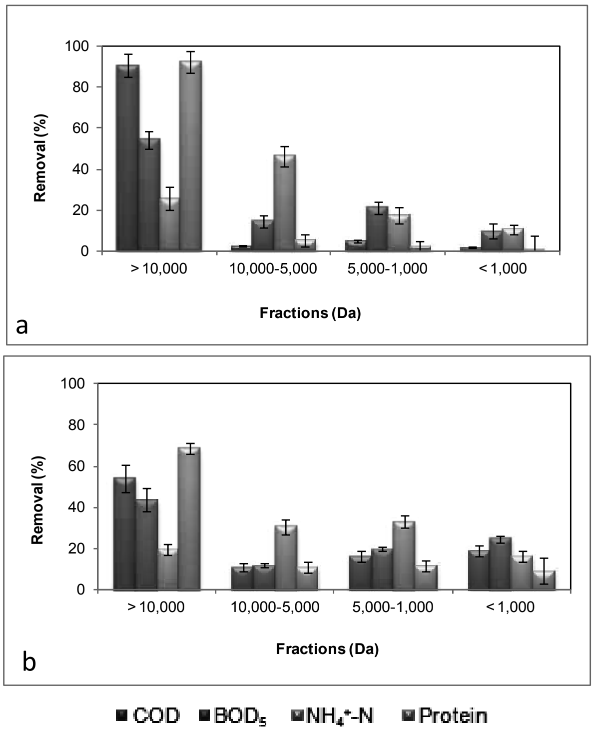
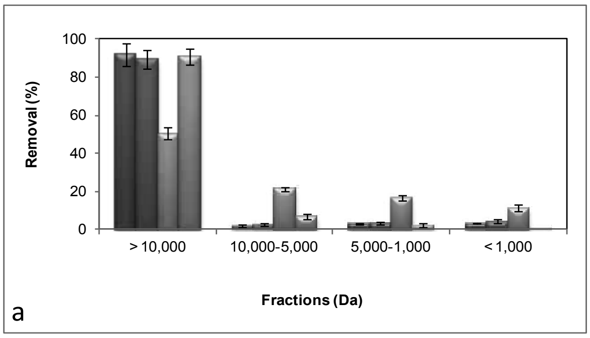



***Figure 8**. Ultrafiltration results of wastewater for stage VII (a) influent, (b) effluent. (Number of replicas: 6)*

### 3.9 Stage VIII

In the influent in stage VIII, the highest retention rates were in the >10,000 Da fraction in all cases, with values of 91%, 90%, 51% and 90% for COD, $BOD_5$, $NH_4^+$-N and proteins respectively (Figure 9). In the effluent, the highest retentions of COD, $BOD_5$ and proteins also occurred in the > 10,000 Da fraction with percentages of 78%, 56% and 80% respectively. For the $NH_4^+$-N, the greatest retention occurred in the <1,000 Da fraction, at a rate of 50%.



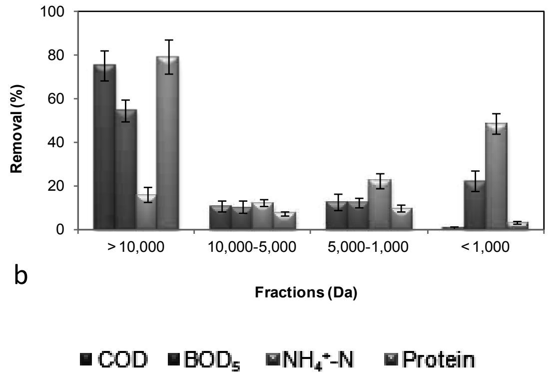




***Figure 9.** Ultrafiltration results of wastewater for stage VIII (a) influent, (b) effluent. (Number of replicas: 6)*

## 4. DISCUSSION

In accordance with previous results, both the washing water and the condensate, as well as their different combinations, suggest that most of the composition of the effluent from stages I to VIII has a molecular weight greater than 10,000 Da. This indicates that extracellular hydrolysis of these compounds is necessary prior to their transport inside the cell and before subsequent degradation by bacterial metabolism. The highest retentions of the four parameters analyzed were principally in the >10,000 Da fraction, where the majority of the macromolecules are located. These polymers are mainly those with a high molecular weight, of which the best known are proteins, carbohydrates, nucleic acid and lipids. Consequently, the proteins showed high retention rates in the >10,000 Da fraction for all influents generally with percentages above 90.

These results are important because according to Sophonsiri and Morgenroth, 2004, the nutrient removal processes are generally limited by the availability of organic matter to denitrification and phosphorus removal. Considering this, the organic matter in wastewater, especially industrial wastewater, can be divided into an easily biodegradable fraction and a slowly biodegradable fraction. Within the latter, the >1,000 Da fractions are found. These fractions require extracellular hydrolysis, meaning therefore, that in order to provide a sufficient amount of electron donors, an efficient biological treatment usually requires both fractions. Levine et al., 1991, established that the hydrolysis of organic materials present in industrial wastewater can be described from a biochemical point of view as three different processes in accordance with the three major groups of organic compounds in water: hydrolysis of carbohydrates, hydrolysis of proteins and hydrolysis of fats. Consequently, the end result of enzymatic hydrolysis is to obtain molecular weight compounds such as sugars, amino acids and fatty acids, which are used as carbon and energy sources for the microorganisms in biological treatment systems.

On the other hand, according to the size distribution in the effluents of stages I and II, although the hydrolysis process occurred, it did not happen on a large scale, since most of the compounds are distributed in the 5,000 to 1,000 dinars range, with some exceptions shown in Figures 2b and 3b. There was however, the presence of compounds of low and high molecular weight, predominantly the latter. In the case of stages III and IV and VI, the highest

proportion of compounds characterized in the effluent were in the <1,000 Da fraction, indicating that there was a high proportion of hydrolyzed compounds, enhancing their incorporation into the bacterial cell, and therefore, their degradation. Finally, for stages V, VII and VIII, the highest proportions are located in the ranges >10,000 and from 10,000 to 5,000 Da, indicating little hydrolysis and compounds of very high molecular weight. These results were consistent with the removal found, where the phases IV and VI had a high efficiency, and thus, low molecular weight fractions in the effluent. The results of removal and the fractionation graphs indicated that the SBR system is particularly useful for the removal of biodegradable organic matter. However, the presence of a high proportion of recalcitrant nitrogen was also found. This is because a maximum retention of approximately 80% for $NH_4^+$-N was only achieved in stages III, IV and VI, indicating that 20% remains distributed in the higher fractions. Such results are consistent with the rates of removal, where a maximum of 70% was reached for $NH_4^+$-N.
According to the SBR treatment system is possible that the reaction stage wich included an aeration and mixing intermittent served to allow the hydrolysis of some compounds and thus the decrease of molecular weights in some stages, situacion that may be due to the growth of microbial poplations of different species which act in different ways on each of the compounds present.

## 5. CONCLUSIONS

In agreement with the analysis of molecular weight distribution, wastewater, and combinations of the two, fractions of >10,000 Da were observed for COD, BOD5, NH4+-N and proteins, indicating that the wastewater evaluated requires extracellular hydrolysis before it can be transported into the cells. In the effluents fractions of <1,000 Da were observed in phases IV and VI. These are the fractions that are incorporated into bacterial metabolism and whose results were consistent with the high removal of organic matter and ammoniacal nitrogen obtained during these stages. SBR system was shown to be capable of allowing the decrease of the molecular weights of the compounds present during some stages of operation of the system, thus indicating a high efficiency and feasibility to operate heavy weight of compounds.

## 6. ACKNOWLEDGEMENTS

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